STRUCTURE OF EPILITHON IN SOME ACIDIC AND CIRCUMNEUTRAL STREAMS IN SOUTH WESTLAND, NEW ZEALAND

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ABSTRACT

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The composition and biomass of epilithon colonising introduced substrata was investigated in two acid (pH 4.3-5.7), brownwater and two circumneutral (pH 6.6-8.0), clearwater streams in South Westland. Scanning electron microscopy showed that epilithon in the circumneutral streams was composed primarily of diatoms (mostly *Gomphonema subclavatum* and *Achnanthes minutissima*) and blue-green algae, whereas in the acid streams other species of diatoms (mainly *Fragilaria* and *Eunotia* species), filamentous algae (including *Stigeoclonium* sp. and *Tribonema* sp.) and deposits of amorphous material (possibly adsorbed or precipitated organic carbon) were common. Energy-dispersive X-ray analysis indicated that silicon was the most abundant element in epilithon at all sites, presumably reflecting the presence of siliceous diatom frustules or mineral particles. Calcium was significantly more abundant (4-30 times) in epilithon from the circumneutral streams, whereas iron was significantly more common in epilithon at one of the acid, brownwater sites. The ratios of organic carbon to chlorophyll a + phaeophytin a concentration were significantly greater at acid than circumneutral sites ($\bar{x}$ = 452 and 197, respectively), indicating that algae comprised proportionately less of the epilithic carbon at the former sites. Adsorbed or precipitated organic carbon appears to be a major component of epilithon in acid, brownwater streams, whereas low algal biomass may be maintained by a combination of nutrient-limitation, growth inhibition by humic substances, and physical abrasion of stone surfaces.

KEYWORDS: epilithon - scanning electron microscopy - energy-dispersive X-ray analysis - acid streams - New Zealand.

INTRODUCTION

Stone surface organic layers in streams (epilithon) are typically composed of an interwoven matrix of slime, fungi, bacteria, algae and fine particulate matter, and represent potentially important food sources for many stream invertebrates (Madsen 1972, Rounick & Winterbourn 1983, Winterbourn *et al.* 1985). A number of factors are known to affect the composition and biomass of epilithon; these include light availability (Steinman & McIntire 1986), flow regime (Scrimgeour *et al.* 1988, Graesser 1988), nutrient availability (Pringle & Bowers 1984, Winterbourn & Fegley 1989), and pH of the water.

Several Northern Hemisphere studies have focussed on the effects of acidification on epilithon. Most of these have revealed increases in algal biomass with decreasing pH (e.g., Hendrey 1976, Muller 1980, Allard & Moreau 1985, Mulholland *et al.* 1986). Reasons for this are thought to include lower grazing pressure by invertebrates coupled with a successional shift to acid tolerant epilithic taxa, and reduced microbial decomposition of algal material (Hall *et al.* 1980, Planas *et al.* 1989). In contrast, Maurice *et al.* (1987) recorded lower biomass of algae in experimentally acidified Michigan streams, apparently as a result of lower nutrient availability and elevated metal (aluminium and/or iron) concentrations. Similarly, Tease & Coler (1984) found

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that algae were absent at low pH in waters acidified by coal leachates. Few workers have investigated responses of other components of epilithon to acidification, although Palumbo *et al.* (1987) studied epilithic bacterial communities in some acidic Tennessee and North Carolina streams, and found that bacterial biomass and productivity were correlated positively with water pH. They suggested that depression of bacterial production at low pH may have been due to chronic effects of H^+ and Al^{3+} ions present in high concentrations, or perhaps to the effects of acidification on the invertebrate grazer community (Palumbo *et al.* 1988).

Little research has been done on epilithon in naturally acidic, running water habitats such as the brownwater streams that are common on the west coast of the South Island, New Zealand. In the present study we investigated the biological and chemical composition of epilithic communities in two acid, brownwater streams and two nearby circumneutral, clearwater streams in South Westland. Our findings are compared with those obtained in studies of anthropogenically-acidified streams in the Northern Hemisphere in an attempt to obtain some insight into the long-term effects of natural acidification on epilithic communities.

MATERIALS AND METHODS

STUDY SITES

Fieldwork was carried out in four small (1.4-2.4 m wide) streams in the Okarito-Franz Josef region of South Westland (170°E, 43°S). Detailed descriptions of the locations, physicochemical characteristics and benthic invertebrate communities of the two acid (Steep Creek and Suspect Stream) and two circumneutral (Hidden Creek and Toilet Stream) sites are given by Collier & Winterbourn (1987).

Climate is humid and mild (average annual temperature 12°C) with very high rainfall that averages 5000 mm y^{-1}. A consequence of the frequent but temporally unpredictable rainfall events is that discharge patterns of most streams and rivers in the region are highly variable. Hidden Creek emanated from a spring and had a stable flow regime compared with the other sites (coefficient of variation (CV) for discharge measured on six sampling occasions = 66% compared with 123, 216 and 223%, respectively for Toilet Stream, Steep Creek and Suspect Stream).

Hidden Creek and Toilet Stream are situated in the Franz-Josef glacier valley and flow into Waiho River. Water pH was typically 6.6-8.0, dissolved organic carbon (DOC) concentration was 0.3-4.7 $g.m^{-3}$, and concentrations of total (< 0.45 μm) reactive aluminium were less than 85 $mg.m^{-3}$. The acid sites drained areas of pakihi that supported a vegetation dominated by ferns (*Gleichenia* spp.), sedges (*Baumea* spp.), rushes (*Juncus* spp.) and manuka (*Leptospermum scoparium* J.R. *et* G. Forst.), and flowed into Okarito River. Water pH was 4.3- 5.7, and DOC and total (< 0.45 μm) reactive aluminium concentrations ranged from 6.6-16.3 $g.m^{-3}$ and 123-363 $mg.m^{-3}$, respectively. Bed materials in all streams were a mixture of cobbles, pebbles and gravels. Toilet Stream and Suspect Stream had closed canopies of vegetation, so little direct sunlight reached their beds, whereas Hidden and Steep Creeks were open to direct sunlight for at least some of the day. They are referred to henceforth as the "circumneutral-open" (Hidden), "circumneutral-closed" (Toilet), "acid-open" (Steep), and "acid-closed" (Suspect) sites, respectively.

EQUIPMENT AND SAMPLING PROTOCOL

On 22-25 January 1985, wire-framed "baskets" covered with 7 mm plastic mesh (900 cm^2, 11 cm deep) were filled with gravel and embedded in the stream beds at the four sites to provide stable attachment points for other experimental equipment. Perspex egg trays were perforated to permit throughflow of water, and were attached to baskets to accomodate experimental substrata (six greywacke river stones and one carbon rod). Trays were covered with thin plastic netting (20 mm mesh) to prevent stones washing away during high flows. Triangular pieces of perspex, each drilled with two holes, were secured to the downstream corners of all baskets to house two scanning electron microscope (SEM) stubs on which greywacke stone chips were mounted with Bostik[R] adhesive. Every two months (January 1985 - January 1986) all stones from the trays and SEM stubs were removed for analysis and replaced with clean (chromic acid washed) substrata.

ELECTRON MICROSCOPY

When stone chips mounted on stubs were removed from the streams they were immediately placed in 3% glutaraldehyde in phosphate buffer and replaced with new stubs on which clean stone chips were mounted. Colonised stubs were later rinsed twice in phosphate buffer, dehydrated in an alcohol series (Rounick & Winterbourn 1983), and air-dried before being coated with 50 nm of carbon/gold palladium. Stone surfaces were viewed with a Cambridge Stereoscan MK II SEM at magnifications of up to 10 000 times. Photographs of representative fields were taken to provide a permanent record.

ENERGY-DISPERSIVE X-RAY ANALYSIS (EDAX)

The elemental composition of epilithic communities at the four sites was determined with an EDAX 9100 system coupled to the SEM. Elements were identified according to the wavelengths of X-rays emitted. The numbers of emissions (per second) provide a semi-quantitative assessment of elemental abundance (Whallon *et al.* 1989).

Carbon rods (5 mm diameter, 10-15 mm long) were attached to trays at the four sites in March and September 1985 (1 rod per site on each date). After about two months (May and November, respectively), the rods were removed, air-dried and stored in dust-proof containers. Before analysis they were mounted on SEM stubs with carbon paste and coated with 50 nm of carbon. Five randomly selected areas on the rod surface that had been oriented upwards in the stream were analysed. Readings were normalised against a blank carbon rod so that only emissions from material which had accrued on rod surfaces since their introduction into the streams were counted. Fields were analysed for 60 (May) or 100 (November) seconds with the system set at 20 kV, 50 μm aperture and spot size 6.

ORGANIC CARBON ANALYSES

Total organic carbon (TOC) was measured using heat-by-dilution dichromate oxidation followed by titration with 1N ferrous sulphate (Maciolek 1962, Collier 1987). Organic carbon concentration was calculated using an oxygen equivalent of 2.86 (Maciolek 1962), and a conversion factor of 1.2 for inefficiency of oxidation (Collier 1987). Results were expressed in terms of total stone surface area (range 20-40 cm^2), determined by wrapping stones in aluminium foil of known weight per unit area.

PHOTOSYNTHETIC PIGMENT ANALYSES

Chlorophyll *a* and phaeophytin *a* were measured on stones that had been frozen following removal from the streams in September and November 1985 and January 1986, and not used for TOC analyses (3 stones on each date). Stones were immersed in 90% acetone (15-50 ml depending on size and shape of the stone) and pigments were extracted overnight in the dark at 4°C. Pigment concentrations were determined spectrophotometrically (Pye Unicam SP 1800) using the method of Moss (1967a,b).

RESULTS

STRUCTURE AND BIOLOGICAL COMPOSITION

Circumneutral Streams

Electron microscopy showed that surfaces of stone chips oriented upwards at the open site were colonised predominantly by the diatoms *Gomphonema subclavatum* (Grun.) and *Achnanthes minutissima* Kutz (Fig. 1A,B), and by a blue-green alga (?*Chamaesiphon* sp.) that often formed a carpet beneath the diatoms (Fig. 1C). The latter was most abundant in the open stream in summer (March and November 1985, January 1986) whereas *Cocconeis placentula* Ehr., a diatom that was usually uncommon, was relatively abundant in July. Other less common diatoms found were *Achnanthes linearis* and a species of *Fragilaria*. Filaments of a cyanophyte, ?*Lyngbya* sp., occurred frequently between diatoms and in crevices, and patches of another unidentified blue-green alga were seen occasionally. Bacteria often occurred on stones in the open stream but fungi appeared to be uncommon. Epilithon at this site was generally free of non-cellular material, but in May and September large amounts of amorphous material were visible (Fig. 1B).

In the closed canopy stream, *A. minutissima*

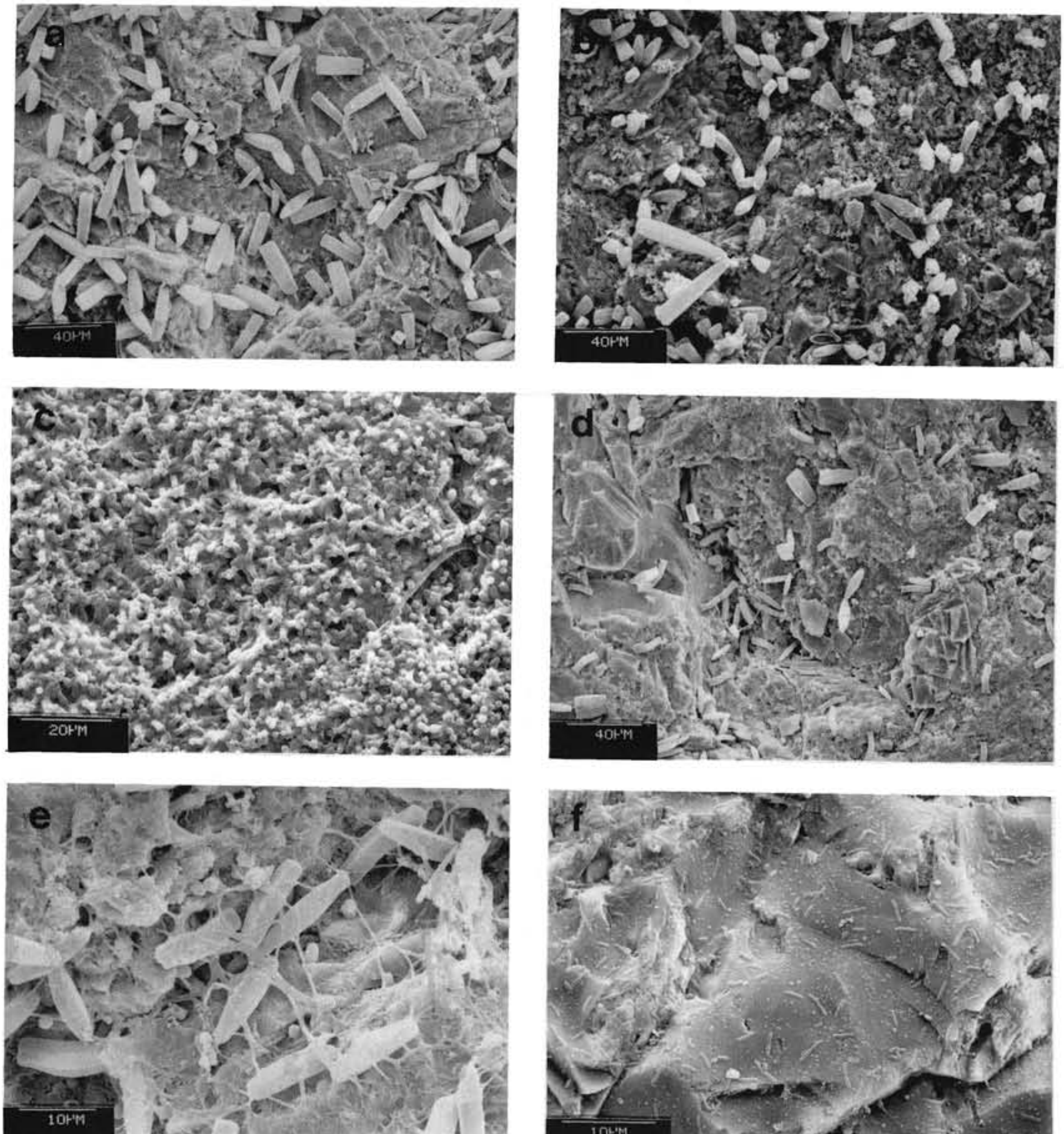

Figure 1. Scanning electron micrographs of epilithon from the circumneutral-open (A, B, C) and -closed (D, E, F) streams in May (B), June (A, D, F) and November (C, E) 1985. Epilithic communities at these circumneutral sites were dominated by the diatoms *Gomphonema subclavatum* and *Achnanthes minutissima* which were more abundant at the open site (A, B cf. D). Diatoms frequently occurred above a layer of blue-green algae (C), and were sometimes embedded in a matrix of mucilage and threads (E). Bacteria were common on stone surfaces (F).

was the most abundant diatom. *Achnanthes lanceolata* (Breb.) Grun and species of *Cocconeis*, *Gomphonema*, *Synedra*, *Fragilaria* and *Eunotia* were less common and often patchily distributed (Fig. 1D,E). ?*Chamaesiphon* sp. was also found at the closed site but, as with the diatoms, it was seen less frequently than in the open stream. Bacteria and filamentous micro-organisms (fungi and/or actinomycetes) were always present on upper stone surfaces in the closed stream, and often they were embedded in a matrix of mucilage (e.g., Fig. 1E,F).

Little amorphous material was incorporated in the epilithon at this site, but in September, accumulations similar to those seen in the open stream on the same date were evident.

Acid Streams

In the open stream, filamentous algae including *Tribonema* sp. (Chrysophyta) and *Stigeoclonium* sp. (Chlorophyta) were usually present on the upper surfaces of stone chips (Fig. 2B). On most dates, only a few isolated strands were seen with the SEM, but in March algal filaments formed a relatively dense covering. Diatoms were not as common in this open stream as at the circumneutral-open site, although *Fragilaria vaucheriae* (Kutz.) Peters and two species of *Eunotia* (*E. curvata* (Kutz.) Lagerst. and an unidentified form) were sometimes abundant, especially in May (Fig. 2A,C). *Fragilaria virescens* Ralfs., *Synedra ulna* (Nitz.) Ehr. and a richly branching, filamentous blue-green alga (?*Tolypothrix* sp.) also occurred at the acid-open site, but were rare. Few bacteria or non-algal filaments were seen on stone surfaces, although rods and spherical objects (possibly bacteria) were noted in May and June, and filamentous micro-organisms were abundant in January. Accumulations of amorphous material (e.g., Fig. 2C) were observed at the acid-open site on most dates and were particularly extensive in May.

The surfaces of stone chips removed from the acid-closed stream in March were covered by a thick layer of mucilage interwoven with fine filaments (Fig. 2D,E). In other months, a few scattered diatoms (mostly *Fragilaria* and *Eunotia* spp.) and filamentous algae were seen, the latter being common in November. ?*Tolypothrix* was only observed in July. Bacteria were never abundant in the acid-closed stream but deposits of amorphous material were common (Fig. 2F).

ELEMENTAL COMPOSITION

Eleven elements were identified in epilithon from the four South Westland streams: silicon, aluminium, calcium, iron, potassium, sulphur, sodium, magnesium, phosphorus, titanium and nickel. Counts for the last five elements were low on rods taken from all sites (0-1 and 0-4 counts per second (CPS) in May and November, respectively). Sulphur counts were also usually low (0-0.6 CPS), but reached 6.6 CPS at the circumneutral-open site in November.

Silicon was always the most abundant element (Fig. 3), presumably because of the presence of diatom frustules and possibly siliceous sand grains that settled on rod surfaces. In May, counts per second for silicon were up to 2.7 times higher on rods from the circumneutral-open stream than on those from any other site, but counts were much higher (up to 3.4 times) at the acid-closed site in November when diatoms appeared to be particularly abundant (see previous section). The other common elements were potassium and calcium (both characteristic of organic material; Winterbourn *et al.* 1985), aluminium and iron (Fig. 3).

Statistically significant differences in elemental counts per second (Kruskal-Wallis, $P < 0.05$) were detected between sites on both dates for all major elements (silicon, aluminium, potassium, calcium and iron). Non-parametric multiple range comparisons (Zar 1974) indicated that epilithon from both circumneutral streams contained significantly ($P < 0.05$) more calcium (4-30 times) than epilithon from either acid site. In contrast, epilithon from the acid-closed site had significantly higher quantities of iron on both dates, and more aluminium and potassium in November, than any other site. In this respect, the elemental profile of stone surface biofilms from Suspect Stream resembled profiles described for epilithon from the most acidic site (pH 4.3) in the Ashdown Forest, southern England (Winterbourn *et al.* 1985).

ORGANIC CARBON AND PHOTOSYNTHETIC PIGMENT

Total organic carbon (TOC) concentrations on stones followed seasonal patterns at most sites (Fig. 4). July minima were recorded in both circumneutral streams (9 and 12 $\mu g.cm^{-2}$), and at the acid-closed site (15 $\mu g.cm^{-2}$), whereas in the acid-open stream TOC values were lowest between May and November (21-26 $\mu g.cm^{-2}$). Maximum TOC concentration at all sites were recorded in either January 1985 or January 1986 and ranged from 21 $\mu g.cm^{-2}$ in the circumneutral-closed stream to 61 $\mu g.cm^{-2}$ at the acid-open site (Fig. 4). Statistically significant differences

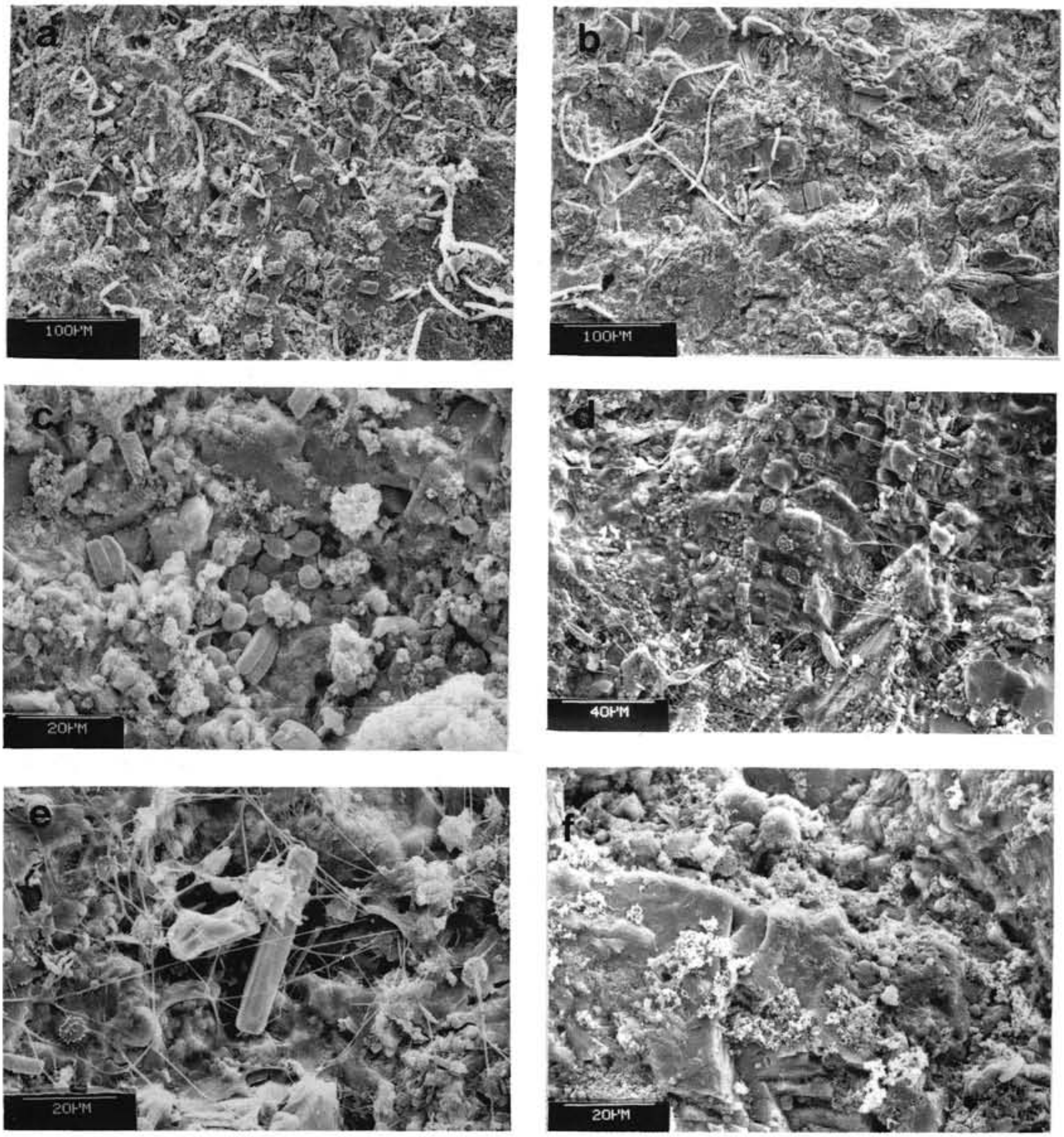

Figure 2. Scanning electron micrographs of epilithon from the acid-open (A, B, C) and -closed (D, E, F) streams in March (D, E), May (A, F) and November (C) 1985 and January 1986 (B). Autotrophs colonising stone surfaces in these streams were mainly the diatoms *Fragilaria* and *Eunotia* and filamentous algae (A, B, C). Mucilage and threads were present on some dates (D, E) and deposits of amorphous material were common (C, F).

in stone TOC concentrations (Kruskal-Wallis, $P < 0.05$) between sites were detected in January and July 1985 when concentrations were greatest in the acid-open stream.

Total pigment concentrations (chlorophyll *a* + phaeophytin *a*) measured in September and November 1985 and January 1986 ranged from 0.08 to 0.47 $\mu g.cm^{-2}$; both extremes were recorded in the circumneutral-open stream (Fig. 4). Mean monthly concentrations at other sites were less variable, particularly at the acid-closed site where they differed by 0.01 $\mu g.cm^{-2}$ or less between sampling dates. Pigment concentrations were significantly different between sites

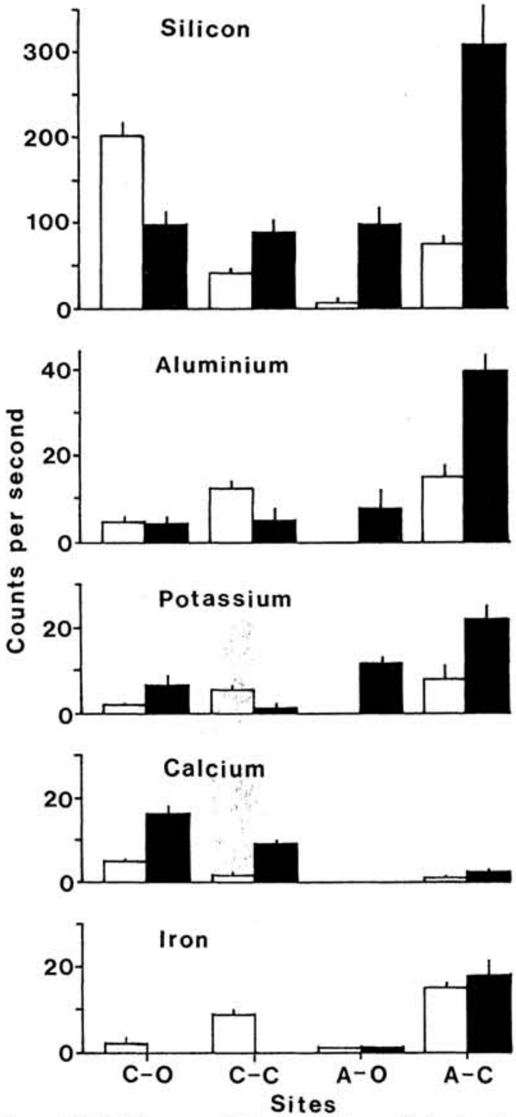

Figure 3. EDAX counts (CPS; $\bar{x} \pm 1$ SE, $n = 5$) for the five main elements in epilithon colonizing carbon rods taken from four South Westland streams in May (open bars) and November (closed bars) 1985. Sites: A-O (acid-open), A-C (acid-closed canopy), C-O (circumneutral-open), C-C (circumneutral-closed canopy). Note the different Y- axis scale used for silicon counts.

only in November (Kruskal-Wallis, $P < 0.05$) when concentrations at both circumneutral sites were greater than in either acid stream. On average, chlorophyll *a* comprised 59-72% of total pigment on stones, but there were no significant differences between sites.

DISCUSSION

Epilithic algal communities in the two types of streams were very different, with diatoms and blue-green algae common at the circumneutral sites, and different species of diatoms and filamentous algae predominating on stones in the acid, brownwater streams. Low pH is believed to affect photosynthetic activity and occurrence of blue-green algae, and to slow down the division rate of many diatoms (Patrick *et al.* 1968, Brock 1973, Patrick 1977, Tease & Coler 1984). Nevertheless, some diatoms live successfully at low pH, and several species of *Eunotia* and *Fragilaria* are typical of acid waters in general (Patrick 1977). Similarly, filamentous green algae such as *Stigeoclonium* are characteristic of waters with high metal concentrations (Stokes 1983). These genera were characteristic colonists of stones at the South Westland acid sites whereas *Cocconeis*, *Achnanthes* and *Gomphonema* were the most abundant genera in the circumneutral streams. Periphytic algal assemblages with these three algal genera among the dominants have been reported widely from non-acid forested and open streams in New Zealand (Biggs & Price 1987, Winterbourn 1990) and elsewhere (Hansmann & Phinney 1973, Patrick 1977, Rushforth *et al.* 1986).

Overall, algal biomass was greater on stones at circumneutral than acid sites with similar degrees of streambed shading, but organic carbon concentration was generally greater on stones at the acid sites. Consequently, organic carbon to chlorophyll a + phaeophytin a ratios (for September and November 1985, and January 1986) averaged 452 in the acid streams but were significantly lower (mean ratio = 197) at the circumneutral sites (Kruskal-Wallis, $P < 0.05$). Another important source of organic carbon on stones at the acid sites is likely to be DOC, which can be incorporated into epilithon by abiotic processes (precipitation and adsorption) or by microbial uptake (Lush & Hynes 1973, Lock & Hynes 1976, Dahm 1981, Rounick & Winterbourn 1983). Most work in circumneutral waters has implicated microbial uptake as the dominant

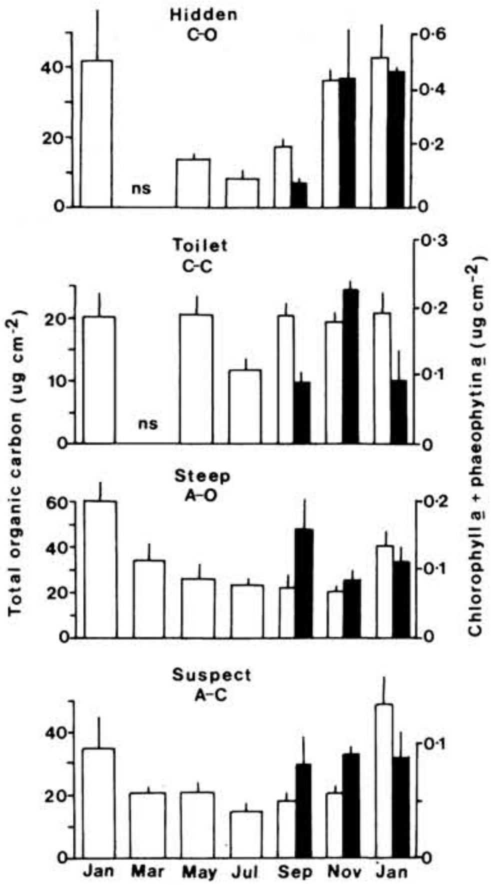

Figure 4. Concentrations ($\bar{x}$ ± 1 SE, n = 3-6) of total organic carbon (TOC, open bars) and chlorophyll a + phaeophytin a (closed bars) on stones collected from four South Westland streams in 1985-86. ns = no sample.

mechanism, but Weber *et al.* (1983) observed large increases in the adsorption capacity of humic acid (an important component of DOC) as pH was lowered from 9.0 to 3.5. They concluded that more humic acid functional groups are in an uncharged state at low pH and hence become more adsorbable. Furthermore, Dickson (1978) found that humic substances settled out of brown lake water at pH 4-5 after addition of aluminium, demonstrating that some metals can increase precipitation rates of humic substances.

Similar adsorption, precipitation and co-precipitation processes may explain the greater abundance of amorphous material on stones taken from the acid streams. Supporting this suggestion is the observation that stones collected from acid, brownwater streams in Westland are typically stained brown, unlike those from circumneutral streams. This colouring disappears following chromic acid digestion indicating that it is produced by organic material. The EDAX data implicate iron and to a lesser extent aluminium as potentially important co-precipitates of organic carbon at the acid sites. The presence of aluminium and manganese in epilithic films at acidic sites in the Ashdown Forest was also taken to indicate the presence of metallo-organic complexes that were established primarily by abiotic processes (Winterbourn *et al.* 1985).

The reasons for observed differences in the abundances of elements among sites is not understood, and relative abundances were not consistent with results reported by Perry *et al.* (1989) for microbially colonised areas of decomposing leaves in some naturally acidic and circumneutral North American lakes. In that study lower concentrations of chloride, potassium, magnesium and calcium were found on birch and oak leaves in acidic lakes, whereas in our epilithon samples calcium occurred in significantly lower, and potassium in significantly higher concentrations in acid streams. Little magnesium was present on stones from any Westland stream, and chloride was not recorded.

The structure and composition of epilithon in naturally acidic, brownwater streams of Westland has some interesting parallels with that in anthropogenically-acidified streams of the Northern Hemisphere. Biological communities in both types of stream are typically dominated by acidophilic species of diatoms and filamentous algae. Furthermore, an increasing body of evidence suggests that adsorbed or precipitated organic carbon is likely to become a more important component of epilithon as acidity increases, particularly where ambient concentrations of DOC are high.

Unlike many acidic, clearwater streams of the Northern Hemisphere, however, algal biomass in acidic, brownwater streams was not characteristically greater than that at circumneutral sites. For example, Parent *et al.* (1986) found 10-15 fold increases in the biomass of periphytic algae in stream channels acidified with H_2SO_4 compared with control channels, and attributed this primarily to higher rates of production and a decrease in grazing activity. In contrast, periphyton densities in the highly acidic (pH 3.6-4.2) and humic Okefenokee Swamp, southern U.S.A., were low compared with those in many other shallow aquatic ecosystems (Schoenberg & Oliver 1988). The authors suggested that the presence of humic acids may be responsible for the low periphyton biomass as they are known to supress algal production and micronutrient uptake. Artificial channel experiments at the acid-open site (Steep Creek) indicated that low pH was not restricting algal biomass (Collier 1988), and suggested that other factors such as low light intensity, variable flow regimes or low nutrient levels may have been limiting. Darkly-stained, humic waters are known to attenuate photosynthetically available radiation (Otto & Svensson 1983, Towns 1985, Bowling *et al.* 1986), but the sampled reaches of our streams were probably too shallow (< 0.25 m in baseflow conditions) for this to have affected the biomass of algae significantly. In short-term experiments, Winterbourn *et al.* (1988 and unpublished data) found that epilithic algal biomass was potentially nitrogen limited in South Westland acid streams, including the acid-open stream of the present study. Furthermore, grazing pressure is unlikely to have been an important regulator of epilithic biomass in these streams as invertebrate densities are low (Collier and Winterbourn 1987, 1990). Nutrient availability, inhibition of growth by humic substances, and frequent physical abrasion of stone surfaces, in combination, may therefore be primarily responsible for maintaining low periphytic biomass in the acidic, brownwater streams of Westland.

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